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14. ABSTRACT

This year, we finally got our p11 knockout mice and were able to conduct the behavioral experiments (Specific Aim 1-3). Here, we will present two sets of new results: first, a control data set that included data from control mice; second, an experimental data set that included data from experimental mice, which received pharmacological treatment (e.g., mice received corticosterone injection) and foot shock exposure. We found the latency to find platform of knockout mice was shorter than that of non-p11 knockout (wild type) mice during the first three training days, although both of their latencies were the same on the final day of training and probe test. This data indicate that p11 knockout in the mice might enhanced learning. We also found that corticosterone resulted in significant decreases in the time in quadrant and number of island crossing in both p11 knockout and wild type control, suggesting that corticosterone induced impairment of memory retrieval, which independent the p11 expression. The ongoing final experiments will allow us to accomplish all of our proposed aims to understand the possible molecular mechanism of p11 in memory retrieval, a molecule associated with PTSD, a devastating disorder, especially in military service members.

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INTRODUCTION

Subject: Posttraumatic Stress Disorder (PTSD) is a chronic and disabling anxiety disorder that occurs after a traumatic event, such as disaster or war. Unfortunately, there is no efficient therapeutic tool for PTSD because of the limited understanding of the pathological mechanism of this disorder. In our proposed study, we examined the possible molecular mechanism, specifically, the role of p11, a calcium binding protein, in memory retrieval in p11 knockout mice with or without stress. We focused on p11 and memory retrieval due to the substantial evidence demonstrating impaired memory retrieval induced by stress and glucocorticoids (1), which can be regulated by p11. P11 is also associated with PTSD.

The most common characteristic of PTSD is the re-experiencing syndrome, when the patient's memory seems to be fixed on a traumatic event whereas the processing of non-trauma-related memories is often impaired. In an animal study, researchers demonstrated that footshock impaired rats' retention performance in a water-maze spatial task, compared to non-stressed controls. This impaired retention performance corresponded to the levels of circulating corticosterone at the time of retention testing. In addition, it showed that the stress-induced retention impairment was blocked by metyrapone, a synthesis inhibitor of corticosterone. Furthermore, systemic corticosterone administered 30 min before retention testing to non-stressed rats induced dose-dependent retention impairment.

Over the last decade, many studies have reported abnormal hypothalamic-pituitary adrenocortical (HPA) axis activity in PTSD. But these studies do not always report changes in the same direction. Both higher and lower concentrations of circulating levels of glucocorticoid in PTSD patients have been reported. For example, Holocaust survivors with PTSD have low urinary cortisol excretion. Another study found high early morning salivary cortisol levels in police officers with PTSD. Therefore, the precise mechanism of glucocorticoid or traumatic stress in memory retrieval performances is unknown. However, and importantly, recent clinical studies have indicated that the administration of high doses of cortisone shortly after experiencing a traumatic event may prevent the development of PTSD, possibly by impairing the retrieval of the traumatic experience.

Hypothesis: In this study, we hypothesized that p11 would play a critical role in mediating the modulatory effects of glucocorticoids on memory retrieval. Our hypothesis is based on the following observations: 1) p11 is expressed in the central nervous system (CNS) and up-regulated by dexamethasone (Dex), a synthetic glucocorticoid; 2) stress increases the induction of p11 in the prefrontal cortex (PFC); 3) p11 over-expression is mediated by glucocorticoid acting via two glucocorticoid response elements (GREs) in the p11 promoter region, indicating that p11 is a possible target for glucocorticoids; 4) p11 mRNA expression is increased in the postmortem PFC of PTSD patients and is also associated with depression.

Purpose: Our immediate objective is to use the p11 knockout stressed animal model, which was developed in our laboratory in collaboration with the Jackson Laboratory, to investigate the role of p11 in memory. Our study will provide an opportunity to determine the possible mechanism of stress-induced changes in retrieval memory, which may be mediated by p11 and glucocorticoids. The information revealed may help in developing new medicines for PTSD treatment. Military personnel who are exposed to trauma at higher-than-average frequencies need to have an efficient medicine to help minimize PTSD. The evaluation of the effect of p11 regulated by glucocorticoid on memory may provide an alternative and/or adjunctive therapy for PTSD in military psychiatry.

Scope:

- **Innovation:** This study will provide information about the molecular mechanism of memory in stress and fill a knowledge gap in current PTSD research. Such knowledge may facilitate the development of novel pharmacological interventions for PTSD.

- **Intervention:** Administration of glucocorticoid agents targeting p11 gene may provide treatment for fear memory in PTSD while minimizing side effects.

- **Application:** Interventions of p11 gene expression have potential for use in both military populations (those on active duty, reservists and veterans) and civilian populations exposed to traumatic stress (natural disasters, vehicle crashes, etc.).

Body

To test our hypothesis in our proposed tasks, **we obtained p11 knockout mice this year and carried out a series of experiments corresponding to our three major tasks:**

1. We examined p11 expression in the brains of wild type.
2. We examined the effects of foot shock on water-maze spatial task performance in p11 knockout and wild type mice. This behavioral experiment determines the role of p11 in memory retrieval.
3. We also examined the role of corticosterone in memory retrieval in p11 knockout and wild type mice. The pharmacological manipulation provided a direct evidence of the role of glucocorticoid in memory in p11 gene deleted mice.

This study is novel because this research has not been done anywhere else. There are no publications about this type of research.

Specific aim 1 all wild type mice were trained for the water-maze spatial task and then divided into 4 groups, each consisted of 10 animals: non-stress control, footshock stress (0.8 mA for 3 times 1 s). 30 min before the retention test trial), corticosterone (3 mg per kg, subcutaneous, s.c.) treated, and saline-treated control. Memory of the water-maze was tested 30 min after the various treatments. There was a probe-trial retention test. In the western blot and immunohistochemistry studies, the mice were sacrificed after verity treatment. Brain tissues were used to examine p11 expression with real-time PCR and Western blot. Two additional groups were sacrificed 30 min after footshock or non-stress control without behavioral testing.

Specific aim 2 used four groups of mice, each consisted of 10 animals: p11 knockout mice + footshock or non-stress control, and wild-type mice + footshock or non-stress control. Footshock and behavior testing was the same as that described in specific aim 1.

Specific aim 3 had 4 groups, each with 10 animals: non-p11 knocked out mice treated with corticosterone (3.0mg per kg, subcutaneous, sic), knockout mice treated with corticosterone, non-knockout mice treated with saline and knockout mice treated with saline control. Footshock procedure and behavior testing was the same as that described in specific aim 1 and 2. Summary of group treatment and test is shown in Table 1.

Specific Aims	**Treatment**	**Number of mice**	**Test**
Specific aim 1	Footshock	10	Water-maze spatial P11 expression
	Non-stress control	10	
	Corticosterone	10	
	Saline control	10	
	Footshock (no behavioral)	10	P11 expression
Specific aim 2	Footshock Non-stress control	P11 KO, N=10 P11 KO, N=10	Water-maze test
	Footshock Non-stress control	Non-KO N=10 Non-KO N=10	
Specific aim 3	Corticosterone	P11 KO N=10	Water-maze test
	Saline	P11 KO N=10	
	Corticosterone	Non-KO N=10	
	Saline	Non-KO N=10	

Apparatus and behavioral methods

Apparatus. All water maze experiments were conducted in a circular tank (120cm in diameter, 50cm deep), located in a dimly-lit room. The pool was filled to a depth of 40cm with water made opaque by adding white non-toxic paint. Water temperature, monitored by a thermometer located 20cm below the water surface, was maintained at 28±1°C by a heating pad located beneath the pool. A circular escape platform (5cm radius) was submerged 0.5cm below the water surface and located in the south-east quadrant. The pool was half surrounded by curtains. The other half was against the wall, which had distinct cues painted on.

Training procedures. On each training day, mice received 4 training trials. On each trial they were placed into the pool, facing the wall, in one of four start locations (north, south, east, and west). The order of these start locations was pseudo-randomly varied throughout training. The trial was complete once the mouse found the platform or 60s had elapsed. If the mouse failed to find the platform on a given trial, the experimenter guided the mouse onto the platform.

Probe test procedures. During the probe test, mice were placed into the pool facing the wall, in the north location. The probe test was 60s in duration.

Quantification of probe test performance. Behavioral data from the probe tests were acquired and analyzed using an automated tracking system. Using this software, the precise mouse location (in x, y coordinates) was recorded throughout the probe test (capture rate 10 frames/s). From this spatial distribution, the following performance measures were calculated automatically:

Time in quadrant time (Q). Amount of time mice searched a virtual quadrant (i.e., 25% of total pool surface area), centered on the location of the platform during training.

Crossings (C). Number of times mice crossed the exact location of the platform (5cm in radius) during the 60s test.

All these measures (or combinations thereof) are used to quantify probe test performance. These methods have been used in over 98% of literature.

Data sets.

The training data *time in quadrant time* and *crossings* were recorded for both p11 knockout and non-p11 knockout mice during four day training period. The differences of Q and C between the two groups were analyzed by two ways ANOVA. Then, a probe test was conducted following the completion of training. Next, we examined the impact of different genetic (p11 knockout and p11 non-knockout), stress (foot shock) and pharmacological manipulations (corticosterone) on water maze performance. For these analyses, probe test data were divided into two data sets. First, a control data set that included data from control mice (i.e., wild-type and p11 knockout mice), pharmacological controls (i.e., mice received control injection of saline), and stress controls (yoked foot shock). Second, an experimental data set that included data from experimental mice (e.g., wild-type or p11 knockout mice), which received pharmacological treatment (e.g., mice received corticosterone treatment) and foot shock exposure. The final data was analyzed by either student t test or one-way ANOVAs.

Summary of Experimental Results.

- Previously we reported that in p11 wild type mice, footshock and corticosterone significantly decreased the time spent on target and produced no effect on time spent on opposite, indicating significantly impaired performance in the water-maze spatial task in stressed mice compared to controls (Fig 1).

- We also reported that in p11 wild type mice, footshock resulted in p11 protein up-regulation, as determined by Western Blot in the hippocampus, cortex and amygdala (Fig 2).

- We found that, in wild type mice, footshock and corticosterone resulted in p11 mRNA up-regulation, as determined by real-time PCR in the hippocampus, a brain region associated with memory function (Fig 3).

- This year, we found the latency to find platform in knockout mice was shorter than that of non-p11 knockout (wild type) mice during the first three training days, although both of their latencies were the same on the final day of training or probe test. This data indicate that p11 knockout in the mice might enhanced learning (Fig 4).

- In this year, we also found that corticosterone resulted in significant decreases of the time in quadrant and number of island crossing in both p11 knockout and wild type control (Fig 5 and 6), suggesting that corticosterone induced impairment of memory retrieval, which independent the p11 expression.

KEY RESEARCH ACCOMPLISHMENTS

- Received knockout mice and footshock protocol approval at USUHS.
- Developed and bred our own p11 knockout mice
- Examined the effect of footshock and corticosterone on learning of memory in both wild type and p11 knockout mice using proposed protocol.
- Examined the effect of footshock on memory retrieval in both wild type and p11 knockout mice using proposed protocol.
- Examined the effect of footshock on memory retrieval in both wild type and p11 knockout mice using proposed protocol.
- Utilized brain tissue collection from p11 knockout mice for Western Blot experiment to determine the role and possible molecular mechanisms of p11 in stress and memory retrieval.

REPORTABLE OUTCOMES

- Oral and poster presentation at the Military Health Research Forum, August 31-September 3, 2009 in Kansas City, MO.

CONCLUSION

Our results indicate that besides the well described effects of stress and glucocorticoids on the acquisition and consolidation processes, stress and glucocorticoids also affect memory retrieval mechanisms. P11 protein expression in the hippocampus, cortex and amygdala, and p11 mRNA expression in the hippocampus in wild type mice were determined. Stress and glucocorticoids resulted in p11 over expression in the mouse brain. The latency to find platform of knockout mice was shorter than that of non-p11 knockout (wild type) mice during the first three training days, although both of their latencies were the same on the final day of training or probe test. Corticosterone also resulted in significant decreases in the time in quadrant and number of island crossing in both p11 knockout and wild type control. Our data suggest that corticosterone induced impairment of memory retrieval, which independent the p11 expression. The ongoing final experiments of Western blot with brain tissue from both wild type and p11 knockout mice will allow us to accomplish all of our

proposed aims and will enable us to understand the possible molecular mechanism of p11 in memory retrieval, a molecule associated with PTSD, a devastating disorder, especially in military service members.

Fig.1 The effect of stress on memory retrieval. Mice had impaired performance in the water-maze spatial task after being given footshock 30 min prior to test compared to control in p11 wild type mice. $p < 0.05$

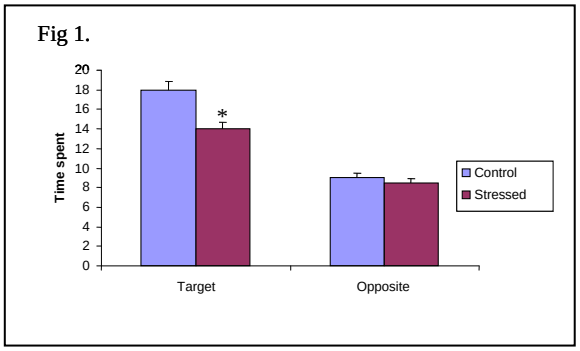
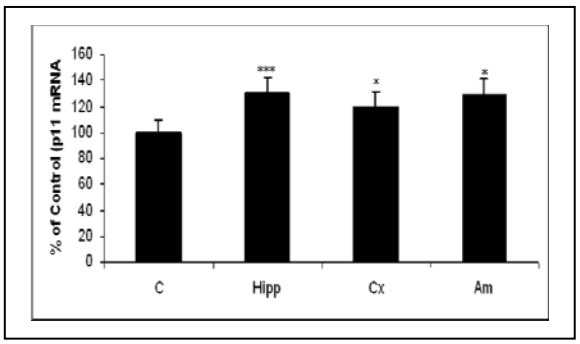


Fig 2. Stress resulted in p11 up-regulation, as determined by PCR in the hippocampus and amygdala of wild type mice. The data were analyzed by Student's t-test; * P<0.05, *** P<0.001. C, Control; Hipp, Hippocampus; Cx, Cortex, Am, Amygdala.



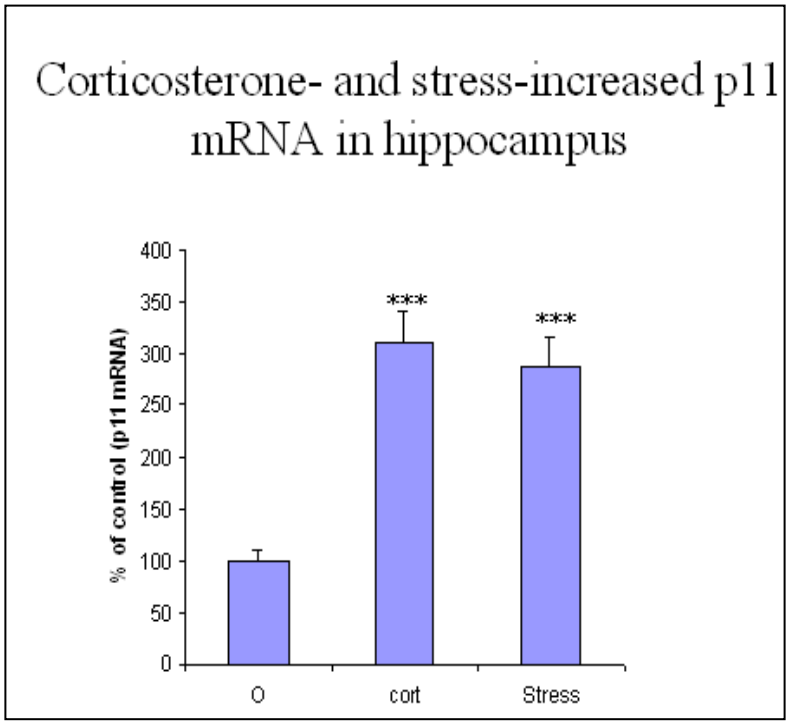


Fig 3. The effects of stress and corticosterone on the p11 mRNA levels in mice hippocampus. Both stress and corticosterone (Cort) increased p11 expression levels in wild type mice hippocampus. The data were analyzed by Student's t-test; $p < 0.05$

Fig 4. The p11 knockout mice had shorter latency to find platform than the non-p11 knockout (wild type) mice had during the training day 1-3. The data were analyzed by two way ANOVA, *p < 0.05; ***p<0.001.

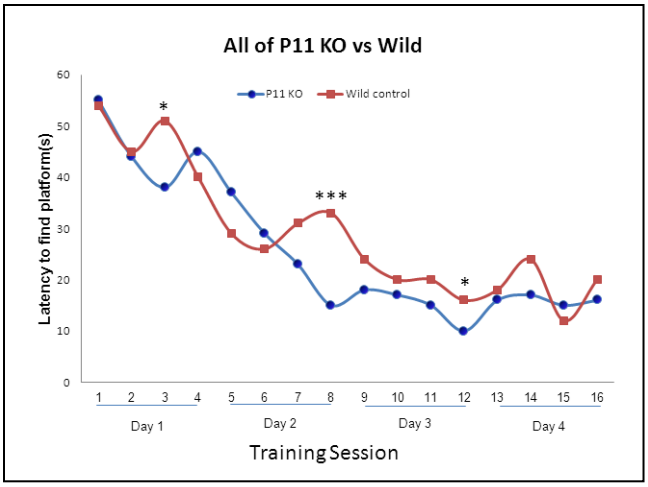


Fig 5. Corticosterone (Corti) significantly decreased time in quadrant (Q) and number of island crossing (C) in p11 non-knockout (wild type) mice. The data were analyzed by student t test, ***p<0.001.

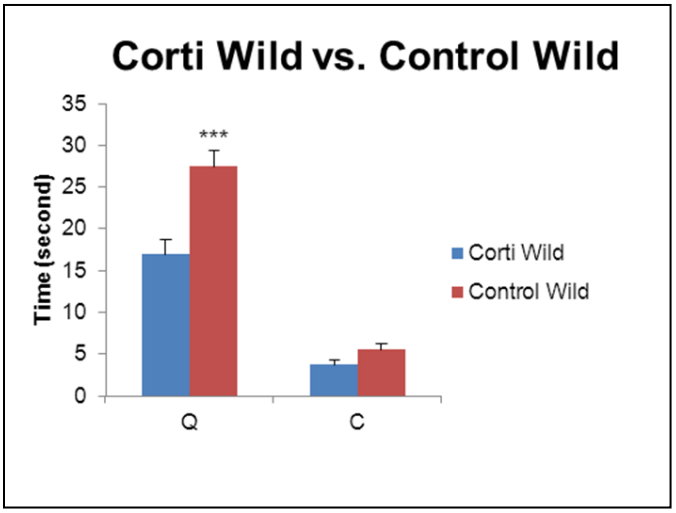
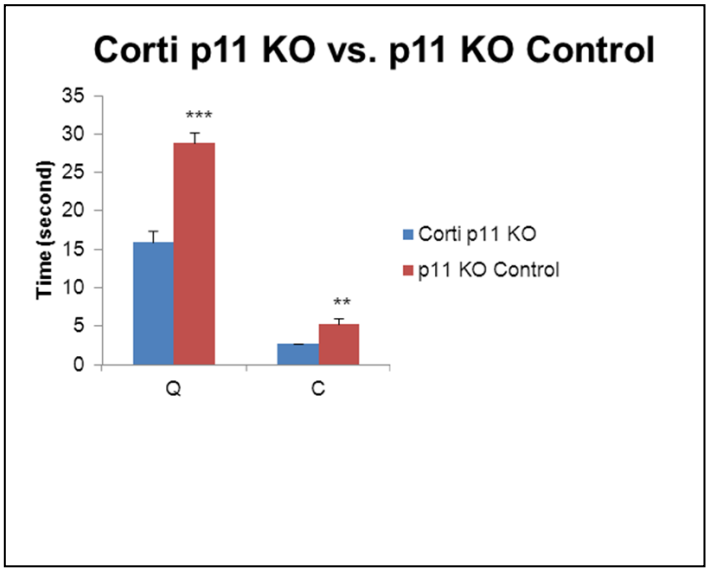


Fig 6. Corticosterone (Corti) significantly decreased time in quadrant (Q) and number of island crossing (C) in p11 knockout mice. The data were analyzed by student t test, **p<0.01; ***p<0.001.



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